

App No. 087700,737
Title: "Humanized Immunoglobulin ..."
Inventors: Paul D. Ponnath, *et al.*

Sequence Range: 1 to 494

5' primer region

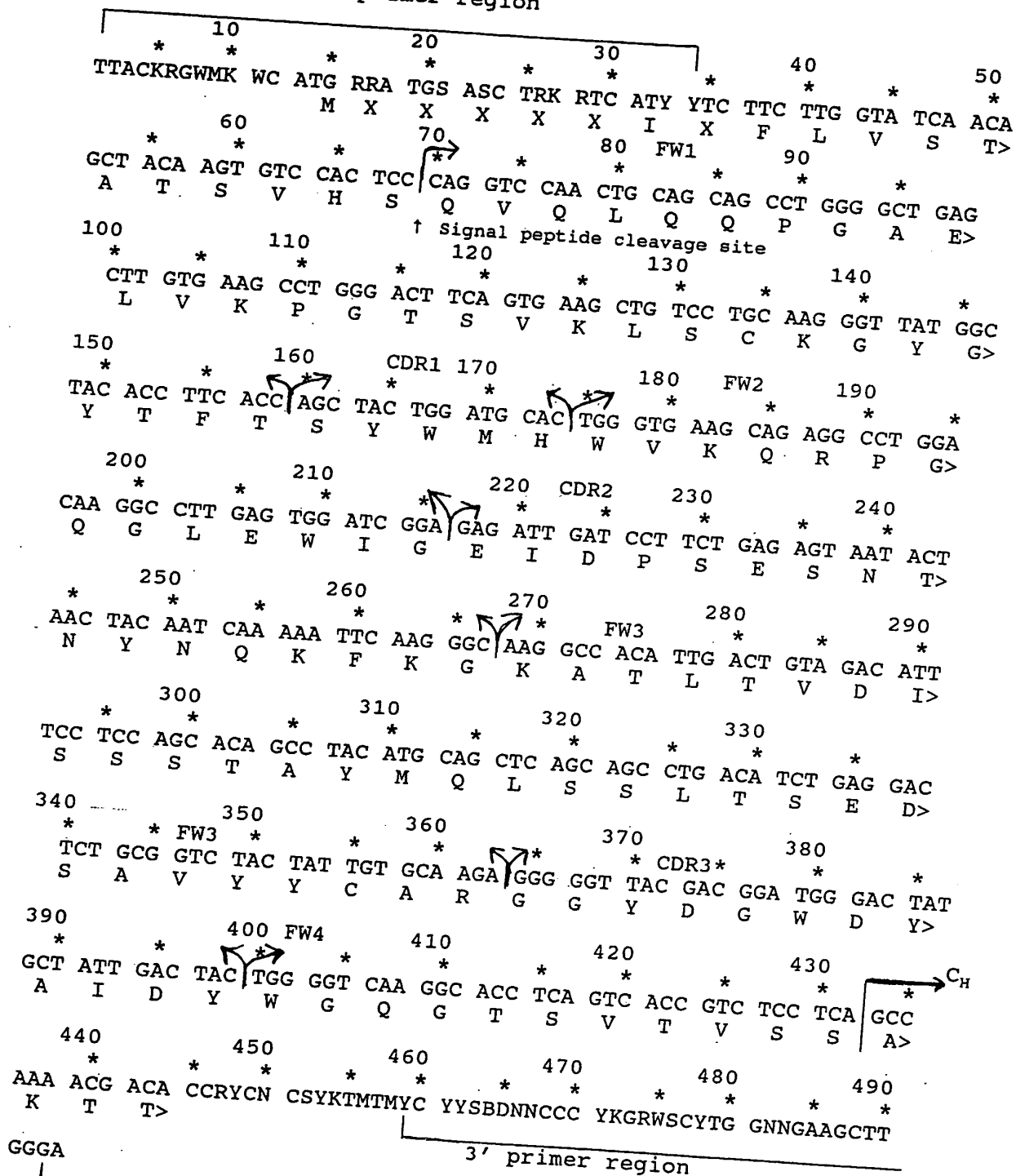
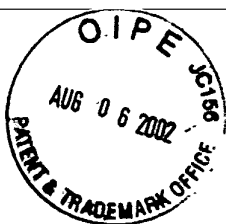


FIG. 1



Title: Humanized Immunoglobulin ...
Inventors: Paul D. Ponath, et al.

Sequence Range: 1 to 428

10 20 30 40 50
* * * * *
TTACTTGACG ACTCGGG ATG GGA TGG AGC TAT ATC ATC TTC TTC TTG GTA TCA
M G W S Y I I F F L V S>
60 70 80 90 100
* * * * *
ACA GCT ACA AGT GTC CAC TCC CAG GTC CAA CTG CAG CAG CCT GGG GCT
T A T S V H S Q V Q L Q Q P G A>
110 120 130 140
* * * * *
GAG CTT GTG AAG CCT GGG ACT TCA GTG AAG CTG TCC TGC AAG GGT TAT
E L V K P G T S V K L S C K G Y>
150 160 170 180 190
* * * * *
GGC TAC ACC TTC ACC AGC TAC TGG ATG CAC TGG GTG AAG CAG AGG CCT
G Y T F T S Y W M H W V K Q R P>
200 210 220 230 240
* * * * *
GGA CAA GGC CTT GAG TGG ATC GGA GAG ATT GAT CCT TCT GAG AGT AAT
G Q G L E W I G E I D P S E S N>
250 260 270 280 290
* * * * *
ACT AAC TAC AAT CAA AAA TTC AAG GGC AAG GCC ACA TTG ACT GTA GAC
T N Y N Q K F K G K A T L T V D>
300 310 320 330 340
* * * * *
ATT TCC TCC AGC ACA GCC TAC ATG CAG CTC AGC AGC CTG ACA TCT GAG
I S S S T A Y M Q L S S L T S E>
350 360 370 380
* * * * *
GAC TCT GCG GTC TAC TAT TGT GCA AGA GGG GGT TAC GAC GGA TGG GAC
D S A V Y Y C A R G G Y D G W D>
390 400 410 420
* * * * *
TAT GCT ATT GAC TAC TGG GGT CAA GGC ACA TCA GTC ACC
Y A I D Y W G Q G T S V T>

FIG. 2

Sequence Range: 1 to 535

5' primer region

10 20 30 40 50

* * * * *

CGATTACTAG TCGAC ATG AAG TTG CCT GTT AGG CTG TTG GTG CTT CTG TTG

M K L P V R L L V L L L

60 70 80 90

TTC TGG ATT CCT GTT TCC GGA GGT GAT GTT GTG GTG ACT CAA ACT CCA

F W I P V S G G D V V V T Q T P>

100 110 120 130 140

CTC TCC CTG CCT GTC AGC TTT GGA GAT CAA GTT TCT ATC TCT TGC AGG

L S L P V S F G D Q V S I S C R>

150 160 170 180 190

TCT AGT CAG AGT CTT GCA AAG AGT TAT GGG AAC ACC TAT TTG TCT TGG

S S Q S L A K S Y G N T Y L S W>

200 210 220 230 240

TAC CTG CAC AAG CCT GGC CAG TCT CCA CAG CTC CTC ATC TAT GGG ATT

Y L H K P G Q S P Q L L I Y G I>

250 260 270 280 290

TCC AAC AGA TTT TCT GGG GTG CCA GAC AGG TTC AGT GGC AGT GGT TCA

S N R F S G V P D R F S G S G S>

300 310 320 330

GGG ACA GAT TTC ACA CTC AAG ATC AGC ACA ATA AAG CCT GAG GAC TTG

G T D F T L K I S T I K P E D L>

340 350 360 370 380

GGA ATG TAT TAC TGC TTA CAA GGT ACA CAT CAG CCG TAC ACG TTC GGA

G M Y Y C L Q G T H Q P Y T F G>

390 region) 400 410 420 430

GGG GGG ACC AAG CTG GAA ATA AAA CGG GCT GAT GCT GCA CCA ACT GTA

G G T K L E I K R A D A A P T V>

3' primer region

440 450 460 470 480 490
 * * * * *
 TCCAT CTTCCACCA TCCAGTAAGC TTGGGAATCC ATATGACTAG TAGATCCTCT
 500 510 520 530
 * * * * *
 AGAGTCGACC TGCAGGCATG CAAGCTTCCC TATAGTGAGT CGTAT

FIG. 3



App No. 08/700,757
Title: Humanized Immunoglobulin ...
Inventors: Paul D. Ponath, *et al.*

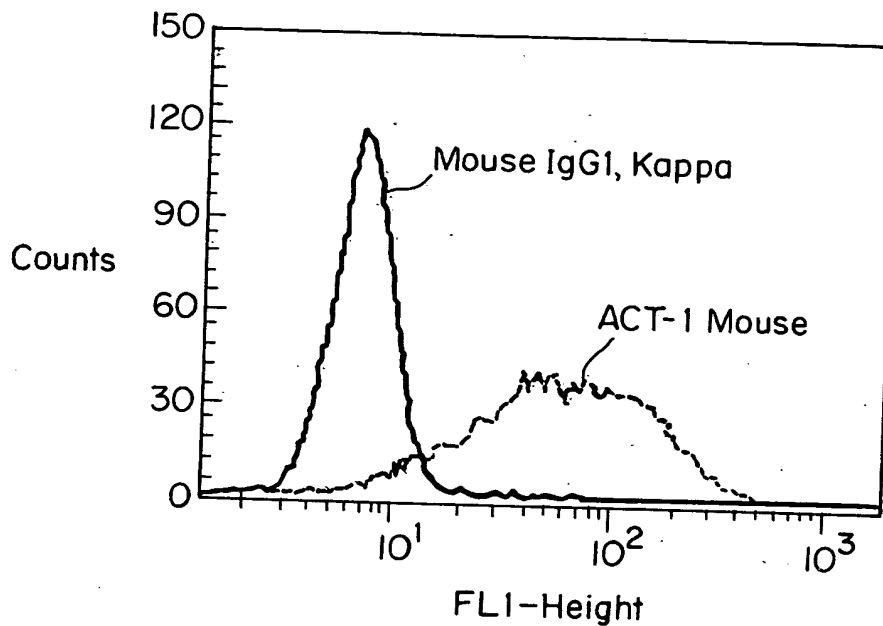


FIG. 4A

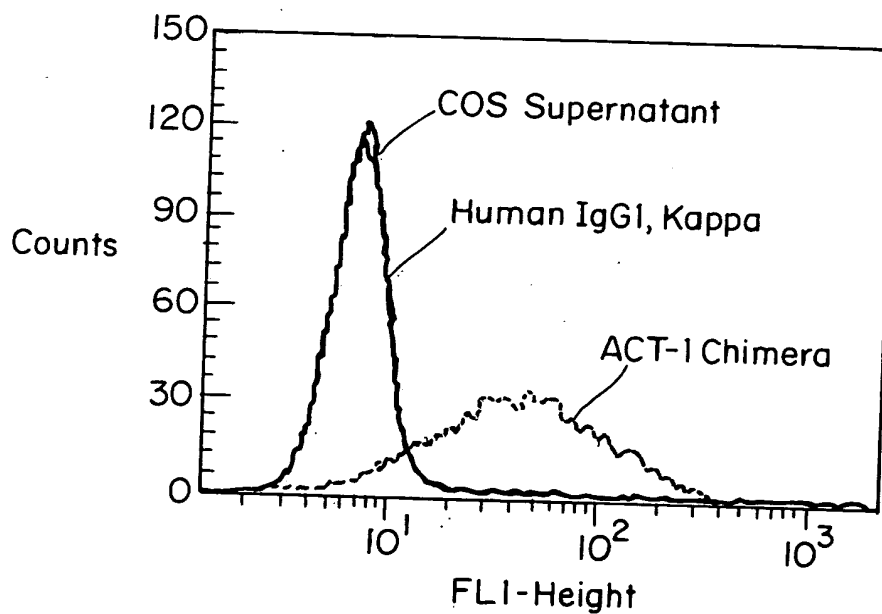


FIG. 4B



Percent similarity: 82.143 Percent Identity: 71.429

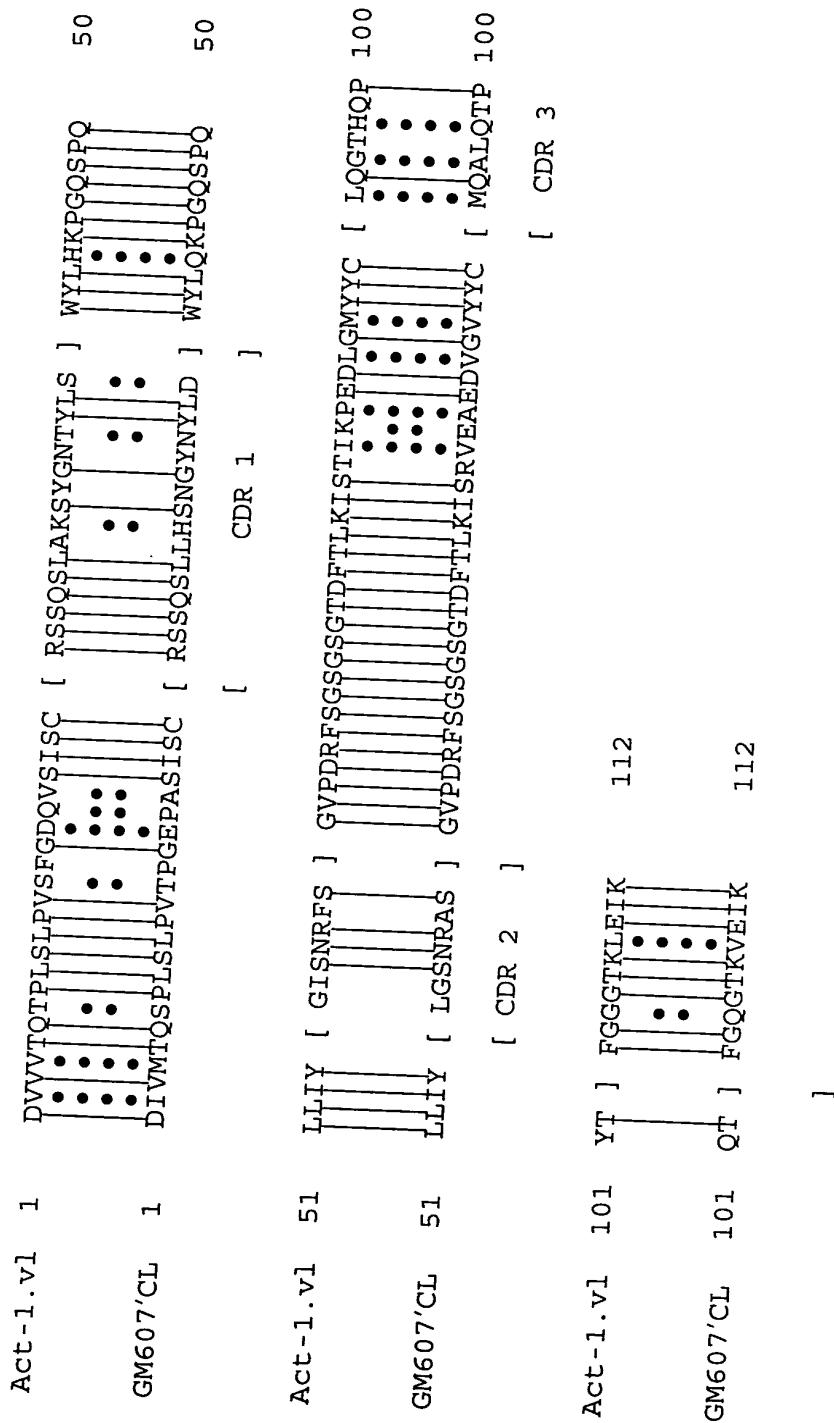


FIG. 5

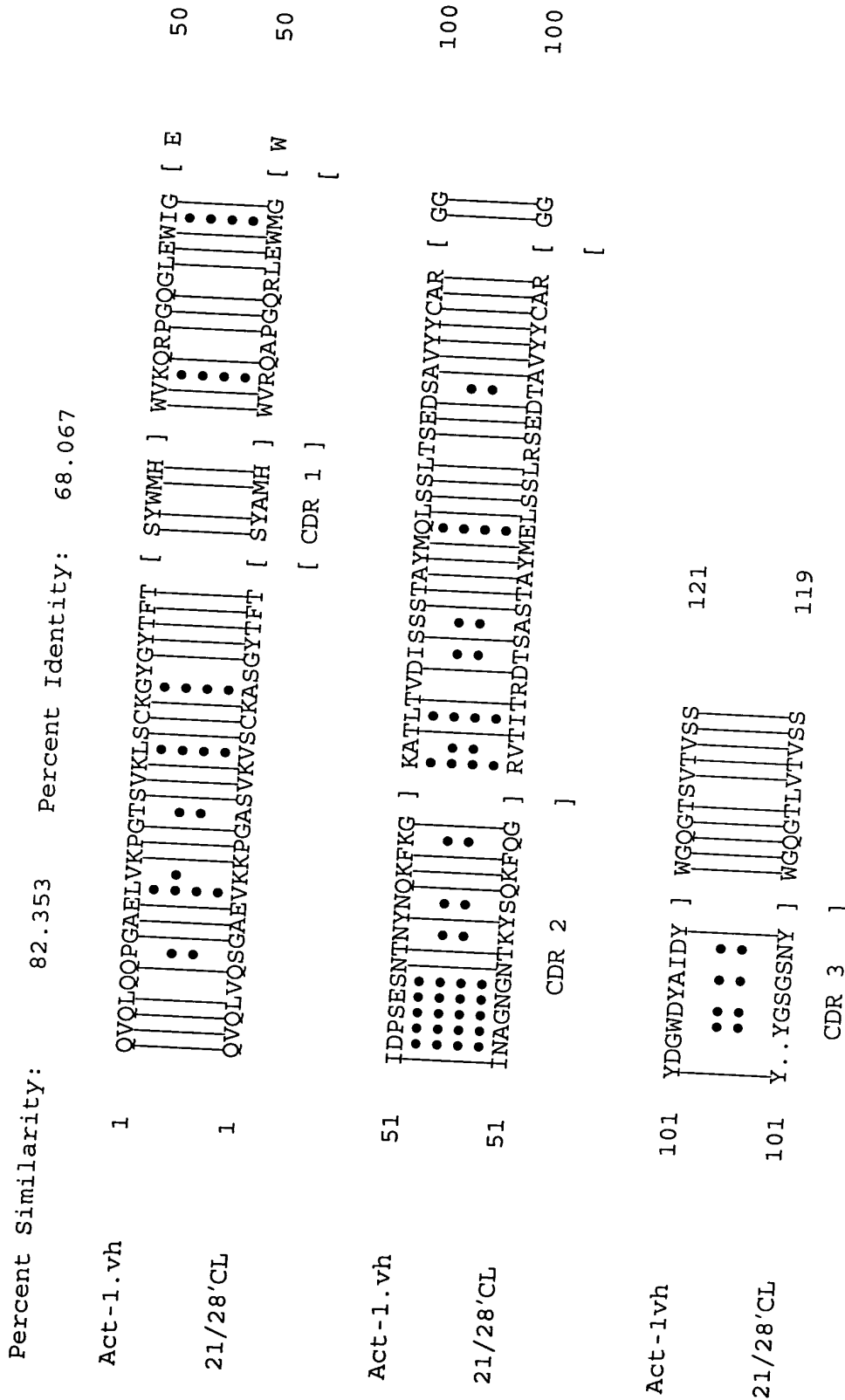


FIG. 6



App No.: 08/700,737
Title: "Humanized Immunoglobulin"
Inventors: Paul D: Ponath, *et al.*

1 ATGAAGTTGCCTGTTAGGCTGTTGGTGCTTCTGTTGTTCTGGATTCTGTTTCCGGAGGT
TACTTCAACGGACAATCCGACAACCACGAAGACAACAAGACCTAAGGACAAAGGCCTCCA 60
[M K L P V R L L V L L L F W I P V S G G]
Signal Peptide
61 GATGTTGTGGTGA CTCAAACTCCACTCTCCCTGCCTGTCAGCTTTGGAGATCAAGTTTCT
CTACAACACCACTGAGTTTGAGGTGAGAGGGACGGACAGTCGAAACCTCTAGTTCAAAGA 120
[D V V V T Q T P L S L P V S F G D Q V S]
Framework 1
121 ATCTCTTGCAGGTCTAGTCAGAGTCTTGCAAAGAGTTATGGGAACACCTATTTGTCTTGG
TAGAGAACGTCCAGATCAGTCTCAGAACGTTTCTCAATACCCTTGTGGATAAACAGAACC 180
I S C][R S S Q S L A K S Y G N T Y L S][W
CDR 1
181 TACCTGCACAAGCCTGGCCAGTCTCCACAGCTCCTCATCTATGGGATTTCCAACAGATTT
ATGGACGTGTTCCGACCGGTGAGAGGTGTCGAGGAGTAGATACCCTAAAGGTTGTCTAAA 240
Y L H K P G Q S P Q L L I Y][G I S N R F
Framework 2
CDR 2
241 TCTGGGGTGCCAGACAGGTTTCAGTGGCAGTGGTTTCAGGGACAGATTTACACTCAAGATC
AGACCCACGGTCTGTCCAAGTCACCGTCACCAAGTCCCTGTCTAAAGTGTGAGTTCTAG 300
S][G V P D R F S G S G S G T D F T L K I
Framework 3
301 AGCACAATAAAGCCTGAGGACTTGGGAATGTATTACTGCTTACAAGGTACACATCAGCCG
TCGTGTTATTTCCGACTCCTGAACCCTTACATAATGACGAATGTTCCATGTGTAGTCGGC 360
S T I K P E D L G M Y Y C][L Q G T H Q P
CDR 3
361 TACACGTTCCGAGGGGGGACCAAGCTGGAAATAAAA
ATGTGCAAGCCTCCCCCTGGTTCGACCTTTATTTT 396
Y T][F G G G T K L E I K]
Framework 4

FIG. 7



1 GATATTGTGATGACTCAGTCTCCACTCTCCCTGCCCCGTCACCCCTGGAGAGCCGGCCTCC
CTATAACACTACTGAGTCAGAGGTGAGAGGGACGGGCAGTGGGGACCTCTCGGCCGGAGG
[D I V M T Q S P L S L P V T P G E P A S 60

Framework 1

61 ATCTCCTGCAGGTCTAGTCAGAGCCTCCTCCATAGTAATGGATCAAACCTATTTGGATTGG
TAGAGGACGTCCAGATCAGTCTCGGAGGAGGTATCATTACCTAGTTTGATAAACCTAACC
I S C][R S S Q S L L H S N G Y N Y L D][W 120

CDR 1

121 TACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGATCTATTTGGGTTCTAATCGGGCC
ATGGACGTCTTCGGTCCCGTCAGAGGTGTCGAGGACTAGATAAACCCAAGATTAGCCCGG
Y L Q K P G Q S P Q L L I Y][L G S N R A 180

Framework 2

CDR 2

181 TCCGGGGTCCCTGACAGGTTTCAGTGGCAGTGGATCAGGCACAGATTTTACACTGAAAATC
AGGCCCCAGGGAAGTGTCCAAGTCACCGTCACCTAGTCCGTGTCTAAAATGTGTCTTTTAG
S][G V P D R F S G S G S G T D F T L K I 240

Framework 3

241 AGCAGAGTGGAGGCTGAGGATGTTGGGGTTTATTACTGCATGCAAGCTCTACCAACTCCT
TCGTCTCACCTCCGACTCCTACAACCCCAAATAATGACGTACGTTGAGATGGTTGAGGA
S R V E A E D V G V Y Y C][M Q A L Q T P 300

CDR 3

301 CAGACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAA
GTCTGCAAGCCGGTTCCTGGTTCCACCTTTAGTTT 336
Q T][F G Q G T K V E I K

Framework 4

FIG. 8



1 ATGGGATGGAGCTGTATCATCCTCTTCTTGGTATCAACAGCTACAAGTGTCCACTCCCAG

TACCCTACCTCGACATAGTAGGAGAAGAACCATAGTTGTTCGATGTTACAGGTGAGGGTC 60
M G W S C I I L F L V S T A T S V H S][Q

Signal Peptide

61 GTCCAACTGCAGCAGCCTGGGGCTGAGCTTGTGAAGCCTGGGACTTCAGTGAAGCTGTCC

CAGGTTGACGTCGTCGGACCCCGACTCGAACACTTCGGACCTGAAGTCACTTCGACAGG 120
V Q L Q Q P G A E L V K P G T S V K L S

Framework 1

121 TGCAAGGGTTATGGCTACACCTTCACCAGCTACTGGATGCACTGGGTGAAGCAGAGGCCT

ACGTTCCCAATACCGATGTGGAAGTGGTCGATGACCTACGTGACCCACTTCGTCTCCGGA 180
C K G Y G Y T F T][S Y W M H][W V K Q R P

CDR 1

181 GGACAAGGCCTTGAGTGGATCGGAGAGATTGATCCTTCTGAGAGTAATACTAACTACAAT

CCTGTTCCGGAACCTACCTAGCCTCTCTAACTAGGAAGACTCTCATTATGATTGATGTTA 240
G Q G L E W I G][E I D P S E S N T N Y N

Framework 2

CDR 2

241 CAAAAATTCAAGGGCAAGGCCACATTGACTGTAGACATTTCCCTCCAGCACAGCCTACATG

GTTTTTAAGTTCCCGTTCCGGTGTAAGTACATCTGTAAAGGAGGTCGTGTCGGATGTAC 300
Q K F K G][K A T L T V D I S S S T A Y M

Framework 3

301 CAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTACTATTGTGCAAGAGGGGGTTAC

GTCGAGTCGTCGGACTGTAGACTCCTGAGACGCCAGATGATAACACGTTCTCCCCCAATG 360
Q L S S L T S E D S A V Y Y C A R][G G Y

361 GACGGATGGGACTATGCTATTGACTACTGGGGTCAAGGCACCTCAGTCACCGTCTCCTCA

CTGCCTACCCTGATACGATAACTGATGACCCAGTTCCGTGGAGTCAGTGGCAGAGGAGT 420
D G W D Y A I D Y][W G Q G T S V T V S S]

CDR 3

Framework 4

FIG. 9



1 ATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCTATTTTAAAAGGTGTCCAGTGTCTAG

TACCTCAAACCCGACTCGACCGAAAAAGAACACCGATAAAATTTTCCACAGGTACACAGTC 60
[M E F G L S W L F L V A I L K G V Q C][Q
Signal peptide

61 GTGCAGCTTGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCC

CACGTGGAACACGTGAGACCCCGACTCCACTTCTTCGGACCCCGGAGTCACTTCCAAAGG 120
V Q L V Q S G A E V K K P G A S V K V S

Framework 1

121 TGCAAGGCTTCTGGATACACCTTCACTAGCTATGCTATGCATTGGGTGCGCCAGGCCCCCC

ACGTTCCGAAGACCTATGTGGAAGTGATCGATACGATACGTAACCCACGCGGTCCGGGGG 180
C K A S G Y T F T][S Y A M H][W V R Q A P

CDR 1

181 GGACAAAGGCTTGAGTGGATGGGATGGATCAACGCTGGCAATGGTAACACAAAATATTCA

CCTGTTTCCGAACCTACCTACCTACCTAGTTGCGACCGTTACCATTGTGTTTTATAAGT 240
G Q R L E W M G][W I N A G N G N T K Y S

Framework 2 CDR 2

241 CAGAAGTTCCAGGGCAGAGTCACCATTACCAGGGACACATCCGCGAGCACAGCCTACATG

GTCTTCAAGGTCCCGTCTCAGTGGTAATGGTCCCTGTGTAGGCGCTCGTGTGCGATGTAC 300
Q K F Q G][R V T I T R D T S A S T A Y M

301 GAGCTGAGCAGCCTGAGATCTGAAGACACGGCTGTGTATTACTGTGCGAGAGGAGGTTAC

CTCGACTCGTCCGACTCTAGACTTCTGTGCCGACACATAATGACACGCTCTCCTCCAATG 360
E L S S L R S E D T A V Y Y C A R][G G Y

Framework 3

361 TATGGTTCGGGGAGCAACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

ATACCAAGCCCCTCGTTGATGACCCCGGTCCCTTGGGACCAGTGGCAGAGGAGT 414
Y G S G S N Y][W G Q G T L V T V S S]

CDR 3 Framework 4

FIG. 10



No.: 08/700,737
Title: "Humanized Immunoglobulin ..."
Inventors: Paul D. Ponath, *et al.*

```

      10      20      30      40
      *      *      *      *
ATG AAA TGC ACC TGG GTC ATT CTC TTC TTG GTA TCA ACA GCT ACA AGT
M K C T W V I L F L V S T A T S>

      Single peptide cleavage site
50      60      70      80      90
      *      *      *      *      *
GTC CAC TCC CAG GTC CAA CTA GTG CAG TCT GGG GCT GAG GTT AAG AAG
V H S Q V Q L V Q S G A E V K K>

100      110      120      130      140
      *      *      *      *      *
CCT GGG GCT TCA GTG AAG GTG TCC TGC AAG GGT TCT GGC TAC ACC TTC
P G A S V K V S C K G S G Y T F>
                                Xba I

150      160      170      180      190
      *      *      *      *      *
ACC AGC TAC TGG ATG CAT TGG GTG AGG CAG GCG CCT GGC CAA CGT CTA
T S Y W M H W V R Q A P G Q R L>
200      210      220      230      240
      *      *      *      *      *
GAG TGG ATC GGA GAG ATT GAT CCT TCT GAG AGT AAT ACT AAC TAC AAT
E W I G E I D P S E S N T N Y N>
                                Nhe I

250      260      270      280
      *      *      *      *
CAA AAA TTC AAG GGA CGC GTC ACA TTG ACT GTA GAC ATT TCC GCT AGC
Q K F K G R V T L T V D I S A S>
290      300      310      320      330
      *      *      *      *      *
ACA GCC TAC ATG GAG CTC AGC AGC CTG AGA TCT GAG GAC ACT GCG GTC
T A Y M E L S S L R S E D T A V>
340      350      360      370      380
      *      *      *      *      *
TAC TAT TGT GCA AGA GGG GGT TAC GAC GGA TGG GAC TAT GCT ATT GAC
Y Y C A R G G Y D G W D Y A I D>
390      400      410      420      430
      *      *      *      *      *
TAC TGG GGT CAA GGC ACC CTG GTC ACC GTC TCC TCA GCC TCC ACC AAG
Y W G Q G T L V T V S S A S T K>
440      450      460      470      480
      *      *      *      *      *
GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG
G P S V F P L A P S S K S T S G>
490      500      510      520
      *      *      *      *
GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG
G T A A L G C L V K D Y F P E P>
530      540
      *      *
GTG ACG GTG TCG
V T V S>

      *Age I

```

FIG. 11



App No.: 08/700,737
Title: "Humanized Immunoglobulin"
Inventors: Paul D. Ponath, *et al.*

```

      10      20      30      40
      *      *      *      *
ATG AAG TTG CCT GTT AGG CTG TTG GTG CTT CTG TTG TTC TGG ATT CCT
M  K  L  P  V  R  L  L  V  L  L  L  F  W  I  P>

      50      60      70      80      90
      *      *      *      *      *
GTT TCC GGA GGT GAT GTT GTG ATG ACT CAA AGT CCA CTC TCC CTG CCT
V  S  G  G  D  V  V  M  T  Q  S  P  L  S  L  P>
      Signal peptide cleavage site

     100     110     120     130     140
      *      *      *      *      *
GTC ACC CCT GGA GAA CCA GCT TCT ATC TCT TGC AGG TCT AGT CAG AGT
V  T  P  G  E  P  A  S  I  S  C  R  S  S  Q  S>

     150     160     170     180 Asp 718     190
      *      *      *      *      *
CTT GCA AAG AGT TAT GGG AAC ACC TAT TTG TCT TGG TAC CTG CAG AAG
L  A  K  S  Y  G  N  T  Y  L  S  W  Y  L  Q  K>

     200     210     220     230     240
      *      *      *      *      *
CCT GGC CAG TCT CCA CAG CTC CTC ATC TAT GGG ATT TCC AAC AGA TTT
P  G  Q  S  P  Q  L  I  Y  G  I  S  N  R  F>
      *Msc I*

     250     260     270     280
      *      *      *      *
TCT GGG GTG CCA GAC AGG TTC AGT GGC AGT GGT TCA GGG ACA GAT TTC
S  G  V  P  D  R  F  S  G  S  G  S  G  T  D  F>

     290     300 NruI     310     320     330
      *      *      *      *      *
ACA CTC AAG ATC TCG CGA GTA GAG GCT GAG GAC GTG GGA GTG TAT TAC
T  L  K  I  S  R  V  E  A  E  D  V  G  V  Y  Y>

     340     350     360     370     380
      *      *      *      *      *
TGC TTA CAA GGT ACA CAT CAG CCG TAC ACG TTC GGA CAG GGG ACC AAG
C  L  Q  G  T  H  Q  P  Y  T  F  G  Q  G  T  K>

     390     400     410 Kas I
      *      *      *      *
GTG GAA ATA AAA CGG GCT GAT GCG GCG CC
V  E  I  K  R  A  D  A  A  P>

```

FIG. 12



App No.: 08/700,737
Title: "Humanized Immunoglobulin ..."
Inventors: Paul D. Ponath, *et al.*

LIGHT CHAIN OLIGOS:		DOUBLE STRAND FRAGMENTS
L1	5'- TTT CCG GAG GTG ATG TTG TGA TGA CTC AAA GTC CAC TCT CCC TGC CTG TCA CCC CTG GAG AAC CAG CTT CTA TCT CTT GCA GGT CTA GTC AGA G	LA
L2	5'- ACT GGC CAG GCT TCT GCA GGT ACC AAG ACA AAT AGG TGT TCC CAT AAC TCT TTG CAA GAC TCT GAC TAG ACC TGC AAG AGA TAG AAG CTG GTT C	LB
L3	5'- CCT GGC CAG TCT CCA CAG CTC CTC ATC TAT GGG ATT TCC AAC AGA TTT TCT GGG GTG CCA GAC AGG TTC AGT GGC AGT GGT TC	LB
L4	5'- ACT CGC GAG ATC TTG AGT GTG AAA TCT GTC CCT GAA CCA CTG CCA CTG AAC CTG TCT GGC ACC CCA GAA AAT CTG TTG GAA ATC	LB
L5	5'- TCT CGC GAG TAG AGG CTG AGG ACG TGG GAG TGT ATT ACT GCT TAC AAG GTA CAC ATC AGC CGT ACA C	LC
L6	5'- ATG GCG CCG CAT CAG CCC GTT TTA TTT CCA CCT TGG TCC CCT GTC CGA ACG TGT ACG GCT GAT GTG TAC CTT GTA AGC AGT AAT AC	LC
HEAVY CHAIN OLIGOS		DOUBLE STRAND FRAGMENT
H1	5'- ATA AGC TTC GCC ATG AAA TGC ACC TGG GTC ATT CTC TTC TTG GTA TCA ACA GCT ACA AGT GTC CAC TCC CAG GTC CAA CTA GTG CAC CGG TTA	HA
H2	5'- TAA CCG GTG CAC TAG TTG GAC CTG GGA GTG GAC ACT TGT AGC TGT TGA TAC CAA GAA GAG AAT GAC CCA GGT GCA TTT CAT GGC GAA GCT TAT	HA
H3	5'- CAA CTA GTG CAG TCT GGG GCT GAG GTT AAG AAG CCT GGG GCT TCA GTG AAG GTG TCC TGC AAG GGT TCT GGC TAC ACC TTC ACC AGC	HB
H4	5'- TAA CCG GTA CTC TAG ACG TTG GCC AGG CGC CTG CCT CAC CCA ATG CAT CCA GTA GCT GGT GAA GGT GTA GCC AGA ACC CTT GCA GGA C	HB
H5	5'- CGT CTA GAG TGG ATC GGA GAG ATT GAT CCT TCT GAG AGT AAT ACT AAC TAC AAT CAA AAA TTC AAG GGA CGC GTC A	HC
H6	5'- TAA CCG GTG TGC TAG CGG AAA TGT CTA CAG TCA ATG TGA CGC GTC CCT TGA ATT TTT GAT TGT AGT TAG TAT TAC T	HC
H7	5'- CCG CTA GCA CAG CCT ACA TGG AGC TCA GCA GCC TGA GAT CTG AGG ACA CTG CGG TCT ACT ATT GTG CAA GAG GGG GTT ACG ACG GAT G	HD
H8	5'- TCA CCG GTG CGG TGA CCA GGG TGC CTT GAC CCC AGT AGT CAA TAG CAT AGT CCC ATC CGT CGT AAC CCC CTC TTG CAC AAT AGT AGA C	HD
H9	5'- CTG GTC ACC GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA G	HE
H10	5'- TCA CCG GTT CGG GGA AGT AGT CCT TGA CCA GGC AGC CCA GGG CCG CTG TGC CCC CAG AGG TGC TCT TGG AGG AGG GTG CCA GGG G	HE

FIG. 13



App No.: 08/700,737
Title: "Humanized Immunoglobulin ..."
Inventors: Paul D. Ponath, *et al.*

RECEIVED
AUG 13 2002
TECH CENTER 1600/2900

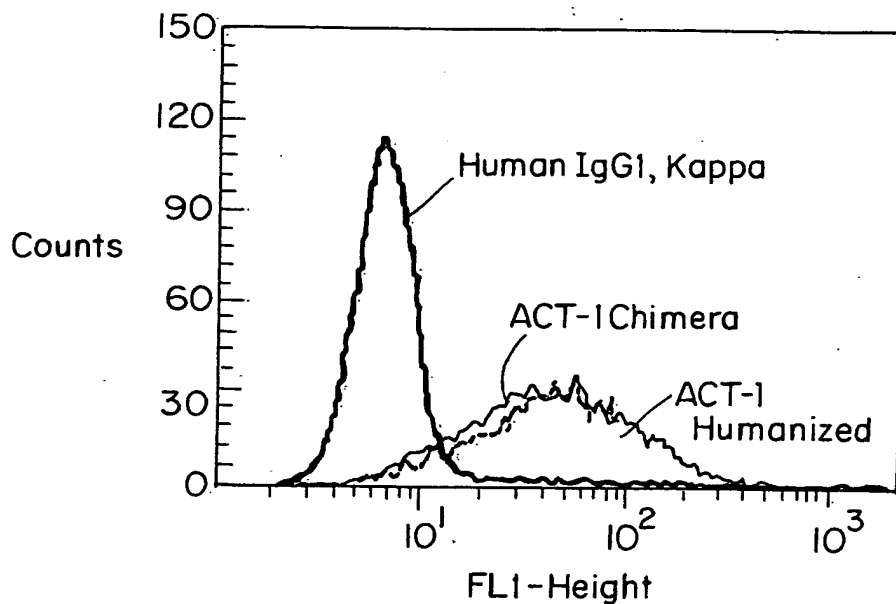


FIG. 14



App. No.: 08/700,737
Title: "Humanized Immunoglobulin ..."
Inventors: Paul D. Ponath, *et al.*

RECEIVED
AUG 13 2002
TECH CENTER 1600/2900

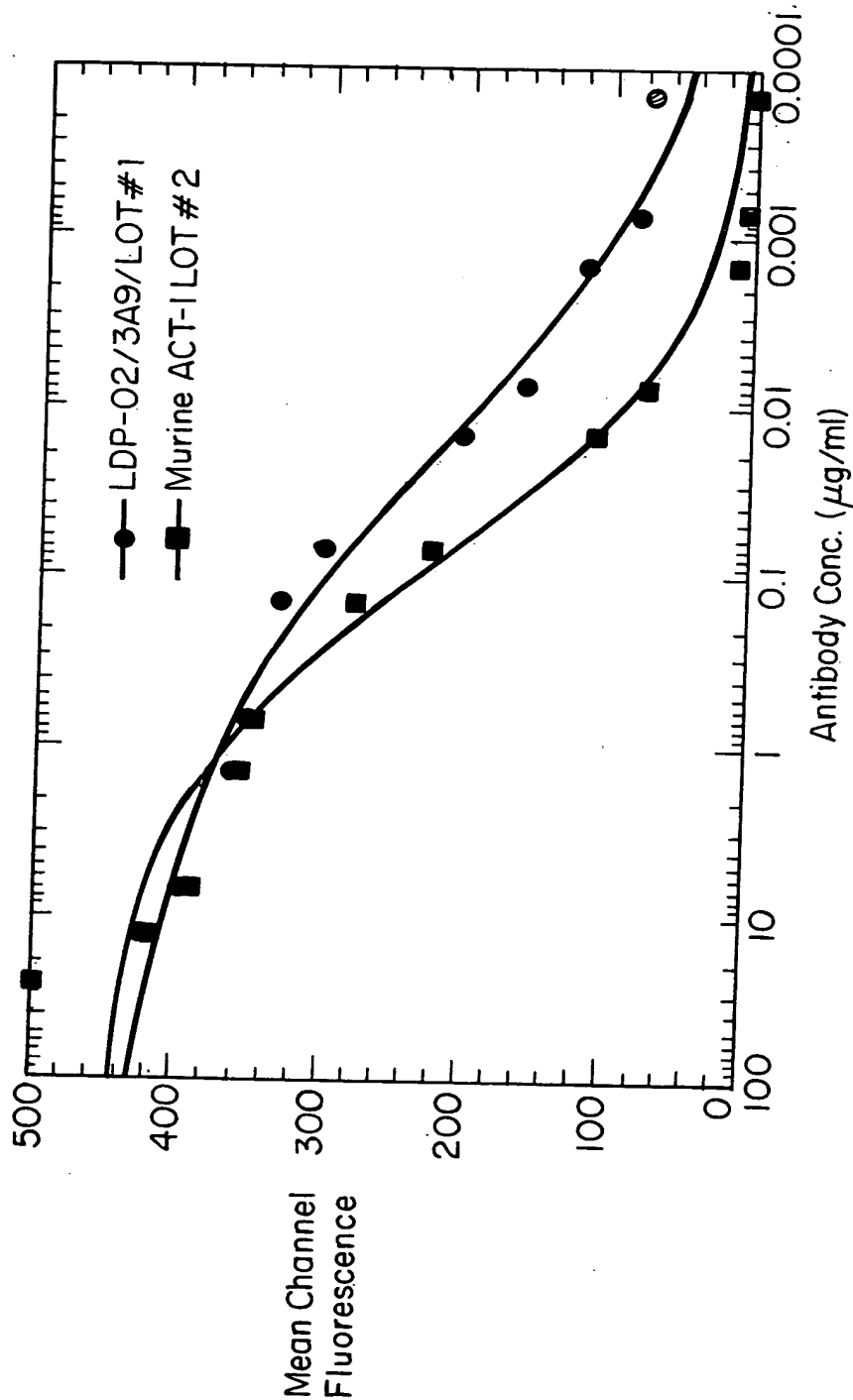


FIG. 15

RECEIVED
 AUG 13 2002
 TECH CENTER 1600/2900

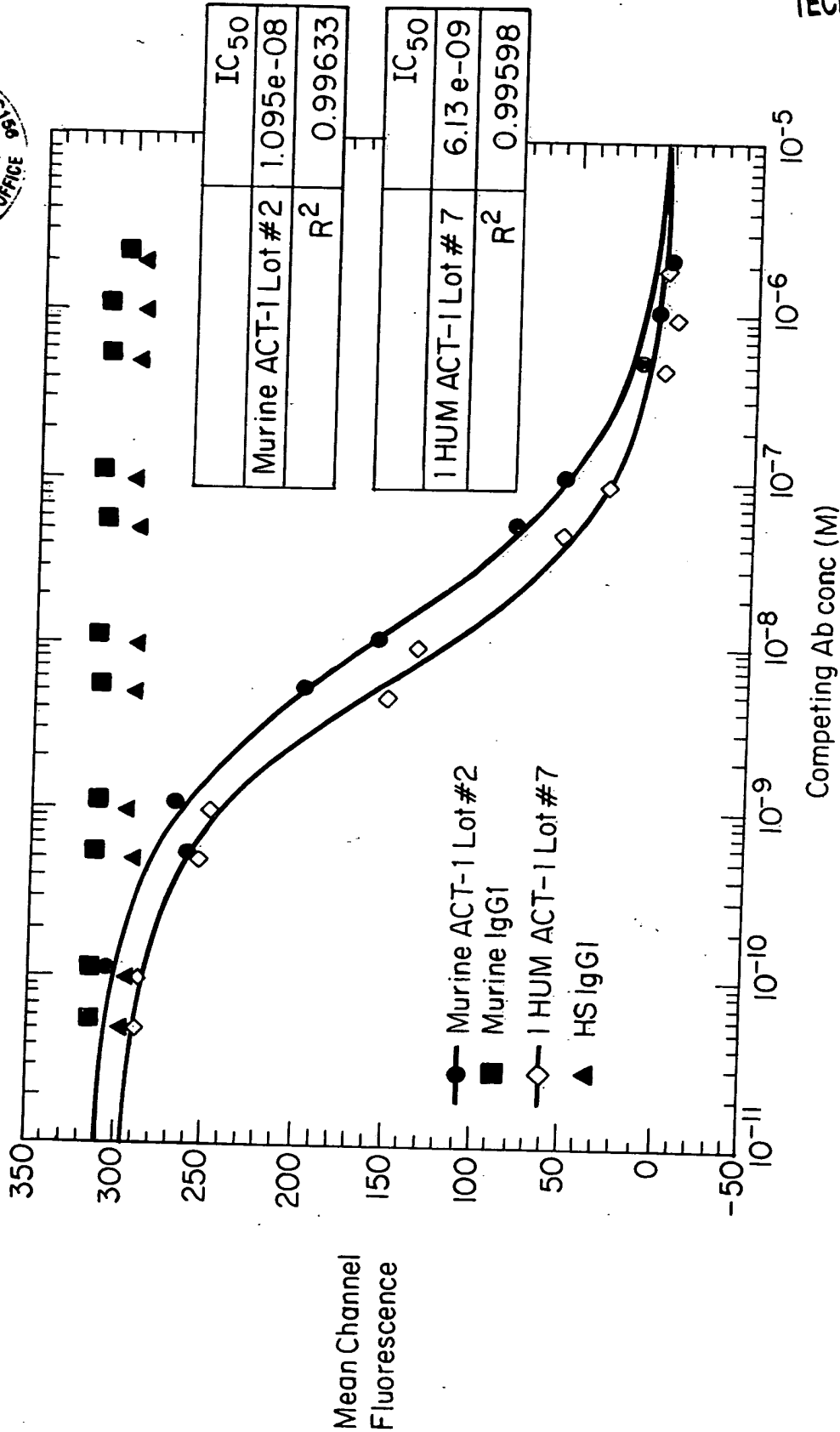


FIG. 16



RECEIVED
AUG 13 2002
TECH CENTER 1600/2900

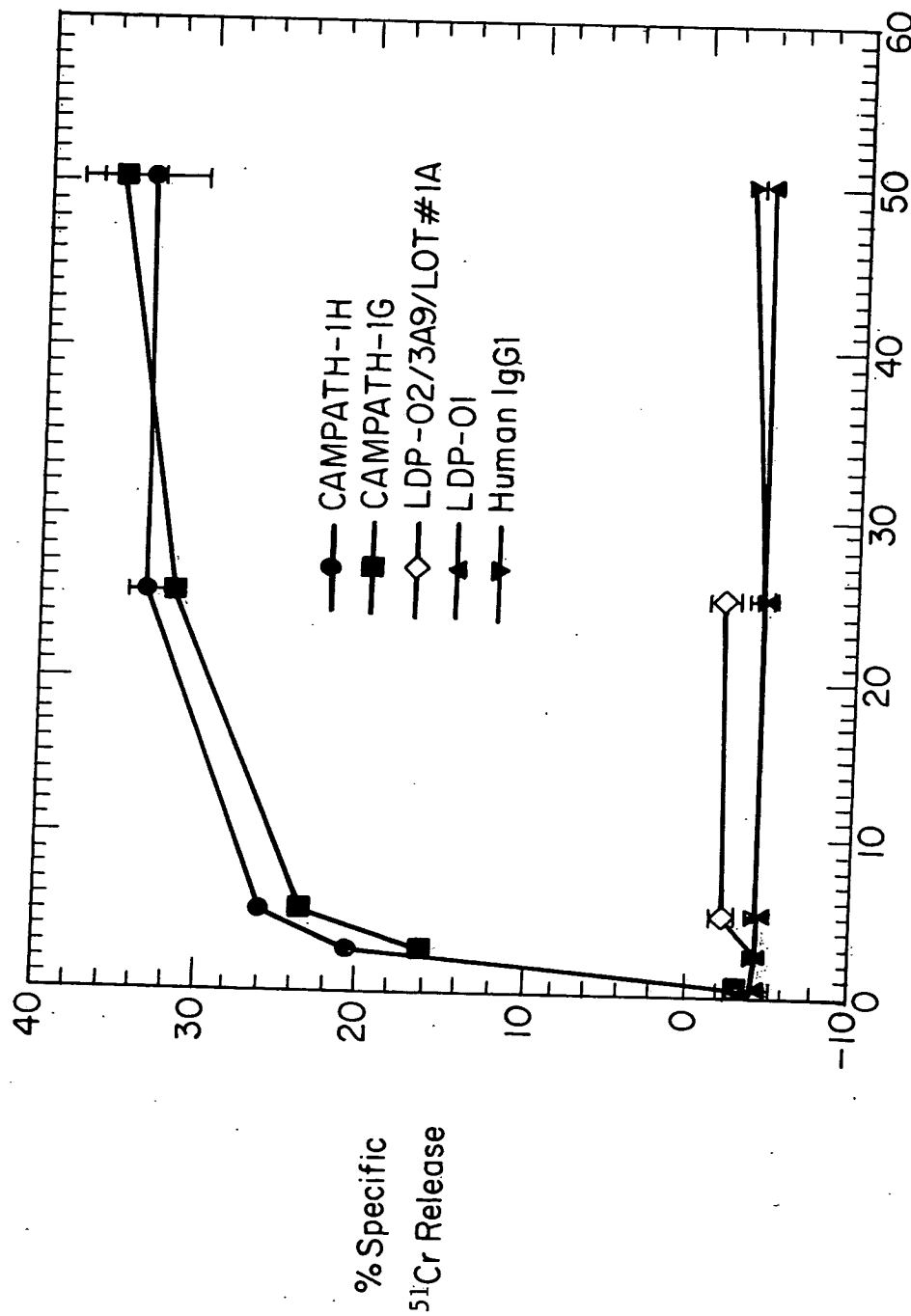


FIG. 17



App No.: 08/700,737
Title: "Humanized Immunoglobulin ..."
Inventors: Paul D. Ponath, *et al.*

RECEIVED
AUG 13 2002
TECH CENTER 1600/2900

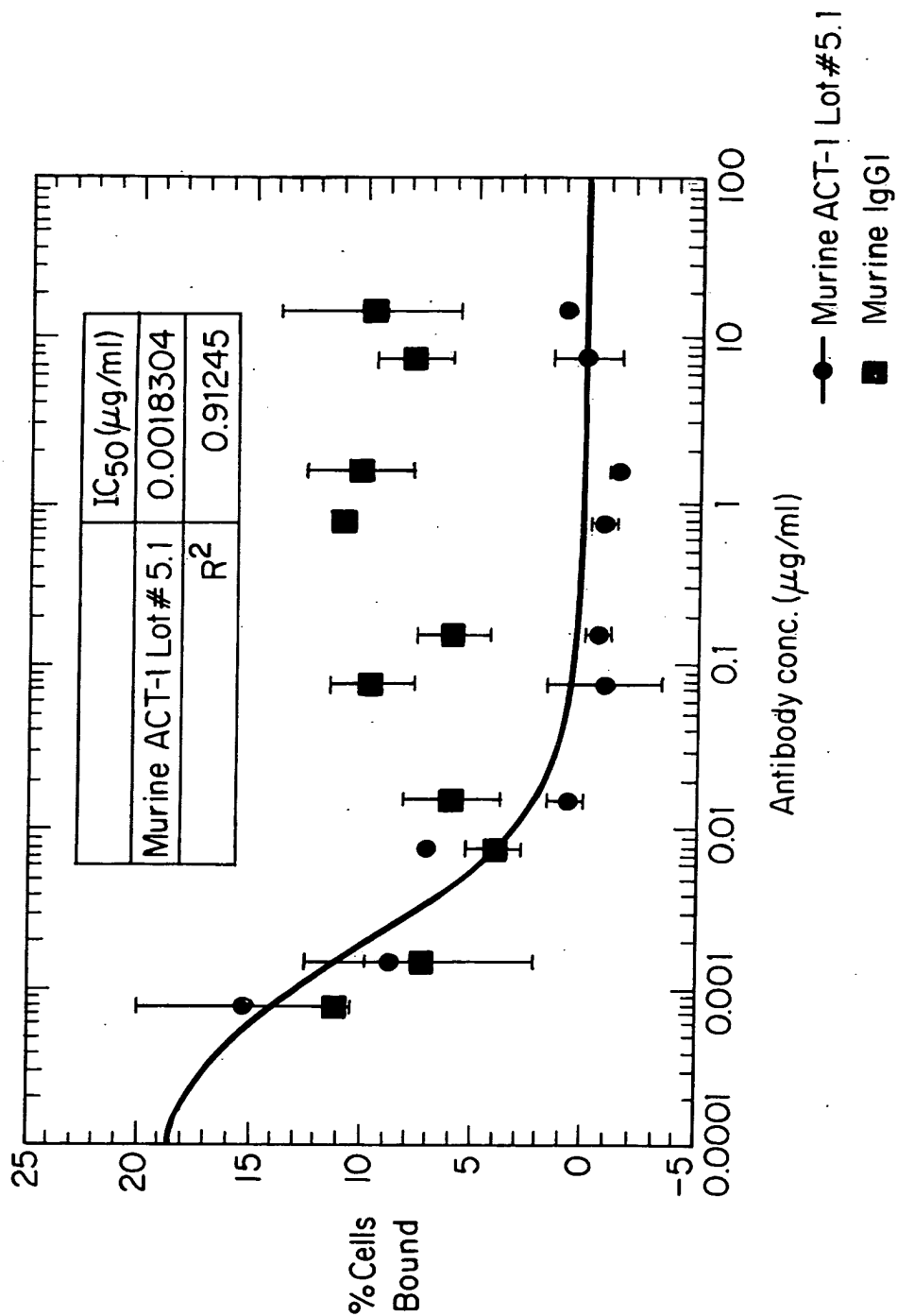
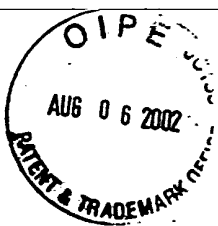
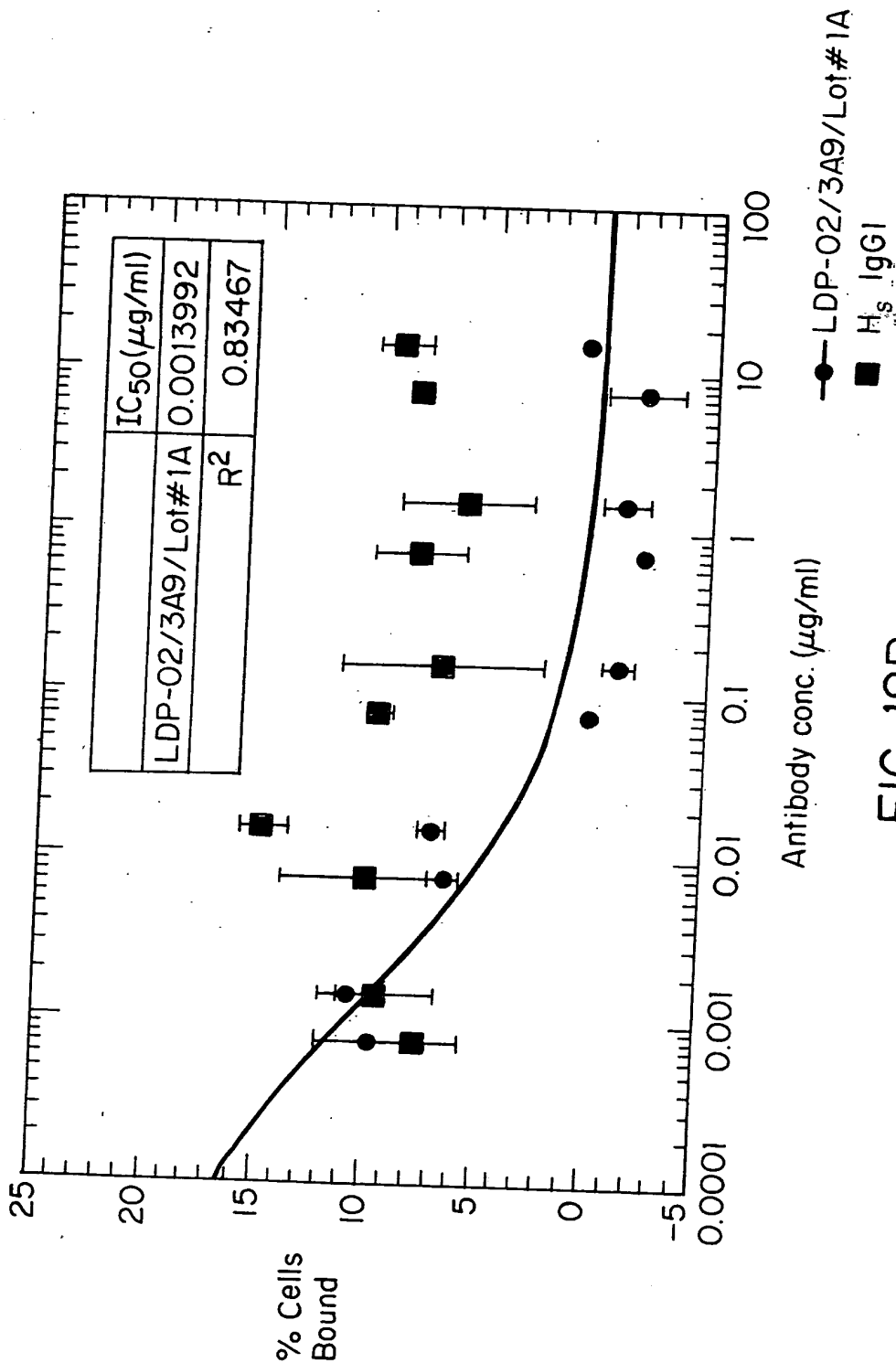


FIG. 18A



App. No.: 08/700,737
Title: "Humanized Immunoglobulin ..."
Inventors: Paul D. Ponath, *et al.*

RECEIVED
AUG 13 2002
TECH CENTER 1600/2900





App No. 08,700,797
Title: "Humanized Immunoglobulin ..."
Inventors: Paul D. Ponath, *et al.*

RECEIVED
AUG 13 2002
TECH CENTER 1600/2900

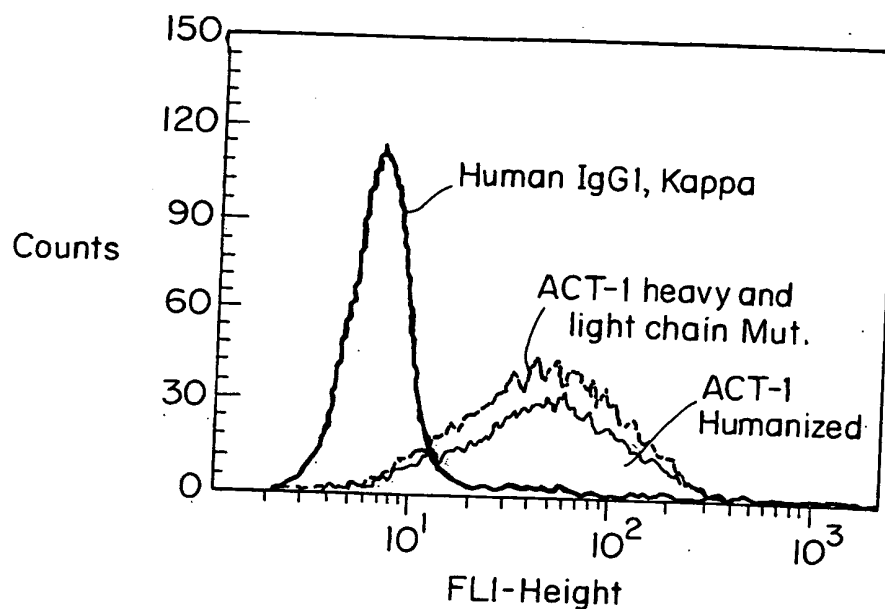


FIG. 19